Cytological changes accompanying secretion in the nectar-glands of Vicia Faba

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(WITH PLATES 10 AND 11)

The cytological changes accompanying secretion have been studied in a number of animal glands with the result that no very obvious degree of uniformity was observed. There are some cases involving the actual destruction of the secretory cells, while in others the integrity of the cell is perfectly preserved. The amount of work on the glandular cells of plants has not been so extensive, yet, among the observations recorded, we find described a considerable diversity in the sequence of events taking place within nucleus and cytoplasm. The nuclei in the case of some glands remain intact but change considerably in their staining reactions, in others the nuclear walls become ruptured and their contents discharged into the body of the cell; the cell-walls themselves in some instances are recorded as being dissolved, and in one case, *Drosera*, the chromatin of the nucleus becomes arranged into a definite number of V-shaped chromosomes during secretion. There are also instances of observers working on similar objects obtaining entirely different results. We are thus forced to recognize the discordant condition existing at present in this field.

Having the above results in view Prof. Francis E. Lloyd kindly suggested to me that I study the cytological changes in the secretory cells of a nectar-gland on the stipules of *Vicia Faba*, the horse bean. It is, therefore, a pleasure to take this opportunity of expressing my indebtedness to Professor Lloyd for his many valuable suggestions and kindly criticism during the progress of this research.

I hoped through the study of these glandular cells to be able to throw some light on the origin of the secretory product and if possible to determine what nuclear and cytological changes accompanied its production and discharge from the gland-cells.

Further, I wished to determine what connection or relation exists between the nuclear and cytoplasmic changes and to find what constituent of the nucleus should it prove to be active in the process is most concerned. The observations described below are designed to attack these questions, though it is recognized that the results rather indicate the need of further careful and exhaustive study than give definite answer to these queries.

METHOD AND MATERIAL

The nectar-glands on the stipules of *Vicia Faba* are beautifully adapted for study; since from the terminal bud to the old mature leaves may be obtained all stages in the development of these glands as well as the entire cycle of secretory action. The living material flourishes in the greenhouse during winter, so that preparations may be made from time to time with little trouble.

Realizing that the methods of fixation and staining in work of this type are open to so much criticism, I have taken all practical precautions against errors from this source. First, observations as far as possible on nuclear positions *et cetera* were made upon free-hand sections of the living glands, and in the second place a large number of fixing and staining solutions were used and the results compared.

The glands were cut from the stipules with a border of non-glandular tissue, thus insuring the presence of all parts of the gland in the preparation, and further facilitating a comparison between gland-cells and ordinary tissue-cells. The material was then, while in the natural condition, quickly placed in the fixing fluids; those employed being Gilson's fluid, picro-acetic, chromo-acetic, picro-corrosive, picro-aceto-sulphuric, alcohol acetic, sublimate acetic, and picro-sulphuric, with the following success. The Gilson's fluid gave most satisfactory preparations, the nucleus and cyptoplasm being in apparently perfect condition. The picro-acetic and chromo-acetic gave fairly good results, and the picro-corrosive used by Reed also proved favorable though inferior to Gilson's fluid. Picro-aceto-sulphuric was rather uncertain, though in some cases fairly good preparations were obtained by its use. Alcohol acetic, sublimate acetic, and picro-sulphuric were all unfavorable. The series for study was then fixed with Gilson's,

picro-acetic, and picro-corrosive, all giving good results and supplying valuable means of comparison. The same precaution was resorted to with the stains, and from the number tried those best adapted to this material were selected.

Auerbach's stain (methyl green and acid fuchsin) was extensively used for studying the cell-contents, as it gives a beautiful differentiation of nuclear substances, and differentiates clearly the chromatic granules of the nucleus from the cytoplasmic granules about the nuclear wall. Heidenhain's iron-haematoxylin with congo red as a counter-stain gave far the clearest preparations and is a most valuable stain, although Reed in criticizing Torrey's results, objects on the ground that it does not differentiate between acid and basic elements of the cell. This is an objection in itself open to criticism, as after the use of fixatives we should not depend on any one stain for such differentiation, and probably Auerbach's stain, which Reed failed to use, is best for such purposes. Torrey, himself (page 429), mentions this same objection to an iron-haemotoxylin and states that on this account he resorted to the use of Auerbach's method for comparison.

Eosin-toluidin blue as recommended by Reed was successfully tried. Eosin and polychrome methylin-blue used separately gave good preparations, while mixed as Romanowsky's stain the results were poor. Throughout this study the artificial conditions of stained preparations were always kept in mind and comparisons continually resorted to so that if errors do exist I believe they should be attributed to other causes.

OBSERVATIONS ON THE LIVING GLAND

The glands are situated on the stipules of the leaves and when mature appear as small dark-red spots, one on each stipule rather near its base.* The immature glands found in the terminal buds are tiny white spots on the pale-green stipules, and the gland is several days old before it becomes colored. They are more conspicuous on the lower or outer surface of the stipule, being less distinct and velvety in appearance on the upper side, the side, namely, on which the secretion occurs. The velvety appearance is due to the presence of the hairs described below. The secretion

* First mentioned by Sprengel, Entd. Geheimniss 357. 1793.

is a limpid watery nectar found usually in the early morning as a droplet on the surface of the gland. This seems to accord with Wilson's observation that these glands may be made to secrete by the stimulus of illumination.

When the gland is examined microscopically it is seen to be perfectly flat, lacking any infolding, and composed of several kinds of cells (FIGURE 1). The inner or lower cells and the epidermal layer seem slightly if at all modified in form, while the epidermal ones are rather more columnar in shape than are those of the general tissue-epidermis. Two kinds of hairs are found on the surface of the glands; one a long slender conical body composed of two cells (FIGURE 2), a small slightly modified basal cell and a long conical apical cell which forms the main body of the hair. This hair is pale-greenish or colorless during life and has a very thick wall. Its function is probably sensitive, though this could not be definitely determined. Haberlandt has suggested that these hairs in another species serve to hold the secretion and prevent its falling off, but I fail to understand, on account of their scarcity on the gland-surface, how they could perform such a function. The other hairs (FIGURE 3) are entirely different in shape, being somewhat club-like, and consisting when mature of five cells. These cells are definitely arranged with a single basal cell usually somewhat smaller than the rest, and four larger cells placed in two pairs one immediately above the other. The four cells might be described as forming the four quarters of an ovate spheroid. These cells contain the secretion-products just as those in other parts of the gland and their color and appearance in life are due to this substance. These will be termed nectar-hairs for the sake of convenience. De Bary states that the glandular properties of these cells have not been observed, but their close similarity to cells of other parts of the glandular area makes it obvious that in younger glands at least these hairs do serve the ordinary secretory functions. The fact that they arise directly from the epidermal gland-cells further suggests their glandular properties.

The color and color-changes in the gland-cells are most interesting. When examining free-hand sections of the living material one finds a brilliant red and a deep blue color arranged in various patterns. At times the epidermal layer with the nectar-hair cells

will be red while the lower cell-layers are blue. Again, layers of red and blue cells may alternate for as many as four or five layers or rows. The contents of the hair-cells are actually at times of different colors, the distal pair being red or blue while the proximal pair are, conversely, blue or red. In mature glands many of the outer tips or distal cells of the nectar-hairs contain a colorless liquid. Thus the cell-contents lose their pigmentation before being thrown out. Often the entire gland is found to be of one color, either blue or red.

This shifting color-scheme suggested to me that possibly the color depended upon the conditions of acidity or alkalinity in the different cells. Therefore experiments were tried in which acid and basic solutions were passed over the glands while on the slide and the responses proved that the color did depend upon the cell's chemical reaction. When a weak HCl solution was passed over a gland containing alternate rows of blue and red cells all the blue cells became red, just as litmus would respond to acid. If dilute NaOH was now applied until the liquid on the slide became alkaline the cells all changed to blue. One is able to alternate this color-change back and forth for a number of times, provided the acid solution is not allowed to remain for too long a time, as the cell-substance is soluble in it, finally dissolving out. This suggests Bonnier's statement that an invertin which dissolves the secretion-products is to be found in glands, and Lloyd also suggested that this invertin might occur in certain non-green cells in the pericycle of *Pteridium* nectar-glands. In the glands of *Vicia Faba* the nuclei in most cases also become red on meeting the acid and thus indicate that they contain material similar to the secretion-substance. On adding the base the nuclei became clear and difficult to see having lost their pinkish acid response.

Testing with Fehling's solution for the variety of sugar present in the secretion substance, cane sugar was found.

HISTOLOGY AND DEVELOPMENT

A. *Structure.* — Studying these glands as stained preparations, one sees very little histological difference between the cells of the gland-area and those in adjacent parts of the stipule. The deeper layers of cells in the gland are strikingly similar to cells of the

same layer in other portions, except for the fact that they rarely contain chlorophyl-granules and that their nuclei seem a little less irregular in form, tending to be as a rule spherical. Lloyd was probably correct in his supposition that the cells lacking chlorophyl in the foliar nectar-glands of *Pteridium* were functional in the secretory process. The epidermal cells of the gland are more columnar in form than those of the general epidermis, being sometimes more than twice as long as broad. The hairs mentioned above are really the only highly specialized structures to be found in these glands. The basal cell of the conical hair (FIGURE 2, *bc*) is often inferior in size to any other cell present, and its contents at times appears very scanty. The tip, or conical cell, of this hair is highly modified, having an enormously thickened wall which in many cases stained diffusely with the methyl green of Auerbach's stain. In size this cell is more than twice the length of any other present, and somewhat broader at its base than the epidermal cells.

The more numerous hairs which for convenience have been termed nectar-hairs, are constructed as described above. The basal cell in this case (FIGURE 3, *bc*) is likewise somewhat smaller than the neighboring cells, but its walls are not so thick as the upper epidermal wall; otherwise it is not remarkable. The outer walls of the remaining four cells of the hair are thin, and the wall forming the distal dome of the hair is very much thinner than elsewhere. The inner partition-walls which separate the four cells are also thin, though usually appearing thicker than the outer ones. As before stated, the contents of these cells is very much like that of the other cells of the gland, except in cases of old glands, when the general gland-cell material stains lightly with the plasma-stains, while these hairs are filled with substances tending to take the nuclear dies. The nuclei of hair-cells (FIGURE 13) are similar to those of other cells, being vacuolated and changing with age in much the same manner.

I consider that the gland proper consists of the club-shaped hairs and the several layers of cells below these, whereas the conical hairs are accessory, being found in no other part of the stipule.

B. *Development.* — Since the general glandular tissue resembles in structure that of other portions of the stipule, little of especial note is expected in its development. The case, however,

of the modified hairs is much more interesting. In very young glands before their contents becomes colored, only the nectar-hair type exists, a few of these being arranged in a central group. As development proceeds new hairs are formed around these as a center until the gland has attained its mature size and color. The nectar-hair is formed in the following manner: the outer wall of an epidermal cell protrudes above the surface to a height about equal to its former depth (FIGURE 4), and its nucleus, which up to this time is still in the resting condition, now divides mitotically (FIGURE 5) into two and thus forms the first cell of the hair. The parent and daughter cell again divide horizontally and so give the hair three cells arranged in a vertical row (FIGURE 6). A vertical division of the two upper, or distal, cells then forms the four-celled arrangement of the mature hair (FIGURE 3). This latter process was not actually observed in my material, but is easily inferred from the stages which do exist.

The conical hairs (FIGURE 2) are found only in glands that have assumed the mature color, and are always far less abundant than the above type. They also arise from an epidermal cell, and in this case the epidermal wall retains its thickness while pushing up to form the heavy cone of the hair. With the development of this type of hair the gland has reached its mature condition.

NUCLEAR AND CYTOPLASMIC CHANGES DURING SECRETION

All observations recorded in these notes were made with Leitz 1/16 oil-immersion objective and number three ocular with the tube-length 170 mm.

(*a*) The youngest glands taken from the terminal buds present the following appearances (FIGURES 7 and 14). The true nucleoli, or plasmosomes, take the acid stains, thus giving with Auerbach's or Congo red a delicate pink color, and they are usually surrounded by large vacuoles. Often two or more plasmosomes are found in the same nucleus (FIGURE 7). They differ slightly in size in the various cells, as do also the vacuoles about them. In some cases the vacuole disappears almost entirely, this being often true of the nuclei in the young nectar-hair cells (FIGURE 13, *A*). The chromatin is granular in structure rarely giving the appearance of a thread-like arrangement. These granules in some nuclei are

closely packed, causing them to stain very heavily, while other nuclei in nearby cells contain much less chromatic substance (FIGURE 12). The nuclei of the gland-cells are somewhat larger and more spherical than those found in the adjacent tissues.

The cytoplasm appears finely granular in structure and is often more or less vacuolated. The cytoplasm in the hair-cells does not differ noticeably from that in other cells of the gland. It is usually densest about the nuclei and never contains any nuclear staining, or cyanophil, material.

(*b*) Considering now a gland that is slightly older or more mature than the former, being the next lower on the stem of the terminal bud. Here are also found (FIGURES 4 and 15) vacuoles of various size about the plasmosomes. The chromatin is again granular as a rule, but many nuclei preparing for division are in spireme stage. In some again the granules are densely arranged while in others the chromatic substance is less abundant.

The cytoplasm is granular in appearance, with numerous vacuoles, and is densest about the nuclei. In a few of the central and therefore oldest nectar-hairs the granules of the cytoplasm have become cyanophil and stain heavily. The walls of these hairs have slightly shriveled. All of the nuclei in the nectar-hairs and the epidermal cells are situated near the cell-center, while in lower layers the nuclear position is not so constant.

(*c*) The cells of the next older gland on the stem are still colorless. The nuclei have large vacuoles about the plasmosomes (FIGURES 8, 9, and 11). The chromatin is granular but more diffuse or not so densely arranged as in the two younger, some nuclei being thin and pale. The nuclei of the nectar-hairs are much poorer in chromatin than those in similar parts of the glands above (FIGURE 13, *B*). The plasmosomes are about equal in size in these three stages of the glands.

The cytoplasm is coarsely granular, being again dense around the nuclei, and contains a few basic staining or cyanophil granules. There is no chlorophyl in any of the cells of these three glands, which appear white in life.

(*d*) The youngest gland containing colored material. The nuclei are vacuolated about the plasmosomes (FIGURE 16), but as a rule those of the nectar-hairs do not contain as large vacuoles

as are found in other parts. The chromatin is arranged much as in the three former cases: in some nuclei the granules are closely packed, in others loosely.

The cytoplasm now becomes denser and more coarsely granular, staining more heavily than in the three former glands. The nucleus is again, as a rule, surrounded by a dense cytoplasmic arrangement. The granules of the general gland-cells take the plasma-stains, while many in the nectar-hairs stain with the nuclear dies.

(*e*) Newly matured glands, slightly older than the above. The nucleus is still vacuolated about the plasmosomes. The chromatin is granular and arranged much as above described, in some instances being closely packed and in others thinly. Some nuclei in the lower cell-layers take the plasma-stains, seeming to have lost their chromatic character. The nuclear positions tend toward the cell-center (FIGURE 10).

The cytoplasm is now rather densely granular, *the granules of the general gland-cells taking the plasma-stains, while those of the nectar-hairs stain with the nuclear stains, particularly the methyl green of Auerbach's stain.*

(*f*) Old but still secreting glands. The nuclei of these glands are slightly shrunken and lie against the cell-walls (FIGURE 17). The vacuole about the plasmosome is not so large and in some is absent; the granular chromatin is as a rule closely arranged. The nuclei in adjacent tissue-cells are often very small, sometimes apparently absent, and in many cases extremely elongate in form. In fact there is a slight difference in all stages between gland-cell nuclei and general tissue nuclei, the former being as a rule, but not always, larger and tending more toward the spherical form.

The cell-contents is at this time particularly interesting in its structure, and in all cases is granular in appearance. The cells of the epidermal and lower layers stain very palely with plasma-stains, while the nectar-hairs stain deeply with nuclear dies such as methyl green, iron haematoxylin, and others. In some cases the granules of the cell stain so densely as almost to hide the nucleus which responds to the stain in exactly the same manner as do these granules (FIGURE 17). The contents of the basal cell of the conical hairs takes the plasma-stains, thus differing in its staining reaction from the nectar-hair cells.

COMPARISON WITH OTHER OBSERVATIONS

Comparing the above changes with those observed in various gland-cells it is difficult to agree in all points with any one author, but on the other hand a study of the results forces one to recognize that secretion in the cell is a process that may be accomplished in many different ways. To quote the statement made in *An American Text-book of Physiology*, on this subject, by Howell: "In the sebaceous secretion the cells seem to break down completely to form the material of secretion; in the formation of mucus by the goblet-cells of the mucous membrane of the stomach and intestines a portion of the cytoplasm after undergoing a mucoid degeneration is extruded bodily from the cell to form the secretion; in the mammary glands a portion of the substance of the epithelial cells is likewise broken off and disintegrated in the act of secretion, while in other glands the material of the secretion is deposited within the cell in the form of visible granules which during the act of secretion may be observed to disappear, apparently by dissolution, in the stream of water passing through the cell. Facts like these show that some at least of the products of secretion arise from the substance of the gland-cells and may be considered as representing the results of a metabolism within the cell-substance. From this standpoint, therefore, we may explain the variations in the organic constituents of the secretions by referring them to the different kinds of metabolism existing in the different gland-cells." This statement relating to animal secretion might with slight alterations be applied equally well to that of plants.

It is now pertinent to consider some of the later contributions to the cytology of secretion among plants and animals and compare the results indicated with the case at hand. Mathews ('99), working on the pancreas cell of various animals, observed the following facts. He found that these cells contained in their basal portions a ball of thread-like fibers which is termed pancreatic mitosome, or "Nebenkern." During secretion these threads, which extend into the upper portions of the cell, break up into the granules which finally form the secretion-substance. The fibrils were found in a number of cases to terminate in, or over, the peripherally placed chromatic masses of the nucleus. Thus Mathews concluded for this and other reasons that the nucleus was passive in the actual

process of *secretion* ("the process of the discharge from cells of their metabolic products"), but was active in the manufacture of the secretion-substance, which process he termed "*hylogenesis*." I feel sure that in the case of the plant gland-cells which I have studied this statement holds equally well. Since no *Nebenkern* or fibers were found to exist in these cells, no direct morphological relation could be established between the nucleus and the secretion-product, although the staining reactions and the occurrence of the granules lead one to think that the nucleus and particularly its chromatic portion is active in the manufacture of the secretion-products. Mathews further finds that during secretion the nucleus moves from the base to the center of the cell, and during rest it returns to a position at the base; thus the nucleus in secretion is passive, merely changing position, due possibly to currents in the cell, but undergoing no structural changes. He states that he finds no trace of a substance of any kind escaping from the nucleus, nor did I observe any such phenomenon in the nectar-glands under consideration. His final position may be seen in this quotation: "I am, therefore, of Nussbaum's opinion, that the changes in the nuclei of secreting cells are passive, and that the nucleus plays no active part either in secretion or zymogenesis."

Harrington ('99) found in the calciferous glands of the earthworm that the disintegration of cytoplasm is in direct ratio to the amount of lime produced, and, as stated in my observations, as the hair-cells become loaded with the secretion-product the cytoplasm of the general gland-cells becomes much paler and therefore more tenuous. At the height of constructive activity, when the cell is ready for the changes which will result in the formation of lime crystals, Harrington finds that the cytoplasm continues to increase in extent, becomes less dense, and vacuoles appear here and there, the nucleus becomes decidedly vesicular, the nucleolus large and densely staining. This description would apply to the cells figured in most contributions on secretion, and in FIGURE 16, which is a gland at the height of secretion, these structures are to be seen.

Schniewind-Thies ('97) studied the nectar-glands in a large number of plants and found that the nuclei of secretion-surfaces are everywhere distinguished from those of the parenchyma by their greater contents of chromatin. She found in secreting cells

that the nucleus sometimes assumed amoeboid shapes and in some entirely disappeared. I find nothing to correspond with these descriptions in my own material, although the slight diversity of form may perhaps be due to amoeboid motion. She further observed that the nucleus in one form lost its response to stains, changing from cyanophil to erythrophil, and as I have recorded above in a few cells of mature glands this staining reaction occurred, but in my material it is rather the exception than a general condition, and was found principally in the deeper layers of the gland, Schniewind-Thies also observed that at the height of secretion the cells are much swollen, which to a less degree is the case in this gland, as is seen by comparing FIGURES 9, 11, and 16 with 17, all drawn at the same magnification.

Huie ('96), studying the changes of the cell-organs of *Drosera*, finds one most noteworthy result; on page 424 she states, "The aggregation of the nuclear chromatin into a definite number of V-shaped segments — eight in *Drosera* — proves that such a change is not a feature of characteristic mitosis, but simply a sign of great activity in the nuclear organs." Similar phenomena have been observed in animal glands, but a constant number of these chromosomes was not found. Huie's observation is striking if the chromosomes do actually exist in a definite number and this number differs from that typical during mitosis. Such would seem to show that chromatin had the power to arrange itself into different sets of chromosomes for physiological and morphological purposes. In my preparations I was unable to find any such chromatic arrangement at any stage of secretion. She also found cytoplasmic changes in staining reactions; during secretion the cyanophil cytoplasm disappeared. The cyanophil chromatin increased during this time and after being thrown out from the nucleus it gave rise to new cyanophil cytoplasm. After long-continued secretion the cyanophil cytoplasm was entirely changed into a scanty erythrophil cytoplasm. This seems to establish a comparable relation between nuclear and cytoplasmic material to that which I have shown in the nectar-glands of *Vicia Faba*. And as Torrey ('02) suggests, a possible explanation of this phenomenon may be that some organic acid is formed during the great metabolic activity of the cell.

Torrey studied the changes accompanying the secretion of diastase in maize and barley. He found as I do that the secretion was intermittent, but held the opinion that the secretion-material was given out as granules from the nucleus through breaks in its wall. The nucleus according to Torrey gradually became loaded with granular material and finally stained a uniform black, the presence of the granules being recognized only by its corrugated edge. The pressure finally bursts the nuclear membrane and the granules stream out in rows, collecting later near the upper, or outer, surface of the cell. This is quite a different process from that recorded by most other writers, and certainly no such nuclear changes occur in the gland which I have studied. Later Reed ('04) has worked on the same material and reached entirely contrary conclusions. In maize he fails to find any indications of granules being given out directly through the nuclear membrane.

Lloyd ('01) investigated the nectar-glands occurring on the leaves of the common brake, *Pteridium*. The position of this gland is similar to that of the one under consideration, and he suggested as the possible function of such organs that the actively secreted sugar may act as a carrier for some other substance in the nature of an excretion. Bonnier had shown that other substances than the secretion-products were thrown off in small quantities from several glands. Lloyd attributed to the cells of the pericycle the power of passing the soluble carbohydrates from the moving sap into the gland, and thus accounted for the greater development of the pericycle. These pericycle cells contain no chlorophyl, and I should rather think that they are actually active in secretion or, in other words, are truly a part of the gland itself; such a view would account equally as well for their greater development in the glandular area.

A felt of dark-colored fungal hyphae was in some cases observed on the surface of the *Pteridium*-glands. I have also found a fungus growth not uncommon on the older glands of *Vicia*. Lloyd offers the apposite suggestion that the nectaral surface is a constant infection-point, the sugary fluid acting as a nutrient medium.

In the case of the nectar-gland under consideration, one is forced to choose the position that *the nucleus takes an important*

part in the manufacture of the secretion-substance, but plays a more or less passive rôle in the essential process of secretion. Further, the cytoplasm also undergoes marked changes during the stages of glandular secretion. It first becomes more vacuolated, and later becomes more coarsely granular, and finally in the nectar-hairs densely granular, at the same time changing its staining reaction from erythrophil to cyanophil. Finally, one must admit that these later changes of the cytoplasm, at least, are largely controlled or influenced by the nucleus and possibly by its chromatic constituent.

SUMMARY

1. The nectar-glands on the stipules of *Vicia Faba* contain rows, or layers, of cells whose contents have different chemical reactions, as is shown in life by their different colors.

2. This difference in chemical reaction indicates very probably a difference in metabolic activity of the cells, since those of definite rows have a similar reaction. The color-response of these cells to acids and bases is the typical litmus change; acids causing the cell-contents to become red, bases changing it to blue.

3. The cells of young glands differ but slightly from the general tissue-cells.

4. The nuclei are granular in structure, often coarsely vacuolated, with one or more plasmosomes surrounded by vacuoles. Their shape tends toward spherical, but in old glands they become shrunken and slightly irregular in form.

5. The position of the nucleus in the secreting cells varies greatly, but is more often near the center of the cell.

6. The nucleus is never observed to give out granular material to the cytoplasm, though evidence is strongly in favor of the supposition that it does transmit substance to the cytoplasm which finally forms, or causes to form, granules and these take during later stages the nuclear stains.

7. In rare cases the nucleus loses its chromatin in older glands and takes the plasma-stains, staining with the acid fuchsin of Auerbach's.

8. The cytoplasm undergoes changes in structure as secretion progresses, at first becoming vacuolar, then slightly granular, still taking the plasma-stains, and then finally becoming densely granular and staining with the nuclear dies.

9. There is evidence to indicate that the cytoplasmic changes are controlled by the nucleus.

10. The nucleus seems to be the center of metabolic activities participating in the formation of the secretion-substance but playing a passive rôle in the actual process of *secretion.*

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Explanation of plates 10 and 11

All figures are from camera sketches. Fig. 1 with Leitz $\frac{1}{6}$ objective and three ocular. Figs. 2, 3, and 6 with $\frac{1}{16}$ objective and 3 ocular. Tube-length 170 mm.

PLATE 10

FIG. 1. Outline of the gland-area, showing the relation of its parts. *ch*, conical hair; *nh*, nectar-hair; *epc*, epidermal cell-layer; *lc*, lower cells.

FIG. 2. Conical hair. *bc*, basal cell; *ac*, apical cell; *n*, nucleus of apical cell.

FIG. 3. Enlarged nectar-hair. *bc*, basal cell; *nc*, nectar-cell.

FIG. 4. Epidermal cell preparing to give rise to the hair; note the up-pushing of epidermal wall and nuclear position. *cw*, cell-wall.

FIG. 5. Similar cell to Fig. 4. in mitosis to give rise to the first cell of the hair.

FIG. 6. Immature nectar-hair, showing the three-cell stage. *bc*, basal cell.

FIG. 7. Nuclear and cytoplasmic structure of the cells in the youngest gland-tissue. *ep*, indicates the position of epidermal cells.

PLATE 11

FIGS. 8, 9, and 11. Immature gland-cells, before their contents becomes colored.

FIG. 10. Mature epidermal gland-cell, shortly after secretion had begun.

FIG. 12. Cells of youngest gland, showing enormous vacuoles and scarcity of chromatin.

FIG. 13. Three stages of the nectar-hair nuclei. *A*, nucleus of very young hair. *B*, nucleus of hair in next gland down the axial stem. *C*, nucleus of hair in gland just becoming mature.

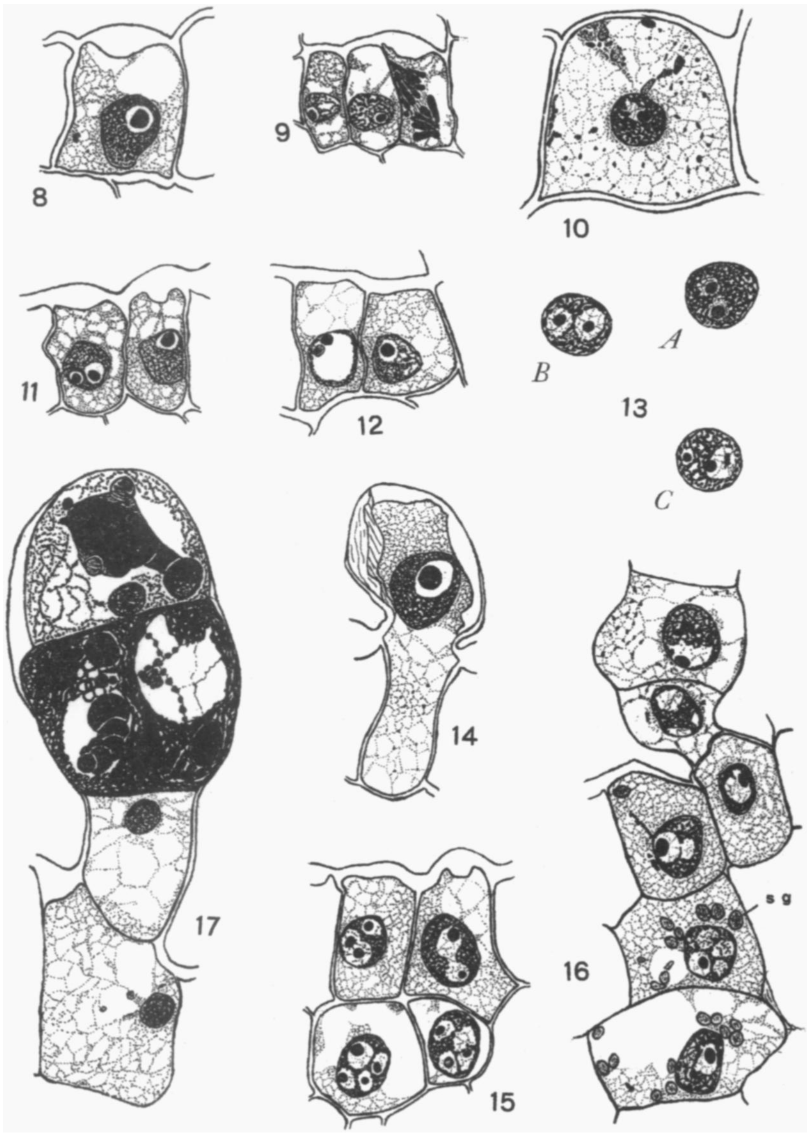
FIG. 14. Youngest gland-cell preparing to form hair, showing cytoplasmic arrangement and nucleus at this stage.

FIG. 15. Cells of immature gland showing numerous plasmosomes in the nuclei.

FIG. 16. Newly matured gland, first formation of large granules in the cytoplasm.

FIG. 17. Secreting hair of an old gland, showing the marked contrast between the staining of hair-contents and of basal and epidermal cell-substance. The hair stains heavily with nuclear dies, so that the nuclei of its cells cannot be certainly determined.

NECTAR-GLANDS OF VICIA FABA



NECTAR-GLANDS OF VICIA FABA